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14. ABSTRACT

Gap junctions (GJ) are conglomerations of cell-cell channels that are formed by a family of 21 distinct proteins, called connexin (Cx)s. The Cxs are transmembrane proteins, which are designated according to molecular mass. They are assembled into GJs through many steps (Figure 1A). Communication through GJs is crucial for maintaining homeostasis [1;2]. Impaired, or loss of, Cx expression has been documented in the pathogenesis of various carcinomas [1;3-5]. Moreover, many studies have shown that over-expression of Cxs in tumor cells attenuates the malignant phenotype in vivo and in vitro, reverses the changes associated with epithelial to mesenchymal transformation (EMT), and induces differentiation [3;4;6]. For example, Cx32 is expressed in the liver, lung, and exocrine glands, and knock out studies have shown that the incidence of carcinogen-induced tumors in these mice is higher [7-9]. Moreover, mutations in several Cx genes have been characterized in inherited diseases associated with aberrant proliferation and differentiation [1;10]. These studies support the notion that Cxs act as tumor suppressors. Despite this the molecular mechanisms by which Cxs are assembled into GJs and how GJs are disassembled are poorly understood.

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Keith R. Johnson, PhD

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Keith R. Johnson, PhD

1. Introduction:

Gap junctions (**GJ**) are conglomerations of cell-cell channels that are formed by a family of 21 distinct proteins, called connexin **(Cx)**s. The Cxs are transmembrane proteins, which are designated according to molecular mass. They are assembled into GJs through many steps (**Figure 1**). Communication through GJs is crucial for maintaining homeostasis [1;2]. Impaired, or loss of, Cx expression has been documented in the pathogenesis of various carcinomas [1;3-5]. Moreover, many studies have shown that over-expression of Cxs in tumor cells attenuates the malignant phenotype *in vivo* and *in vitro*, reverses the changes associated with epithelial to mesenchymal transformation (**EMT**), and induces differentiation [3;4;6]. For example, Cx32 is expressed in the liver, lung, and exocrine glands, and knock out studies have shown that the incidence of carcinogen-induced tumors in these mice is higher [7-9]. Moreover, mutations in several Cx genes have been characterized in inherited diseases associated with aberrant proliferation and differentiation [1;10]. These studies support the notion that Cxs act as tumor suppressors. Despite this the molecular mechanisms by which Cxs are assembled into GJs and how GJs are disassembled are poorly understood.

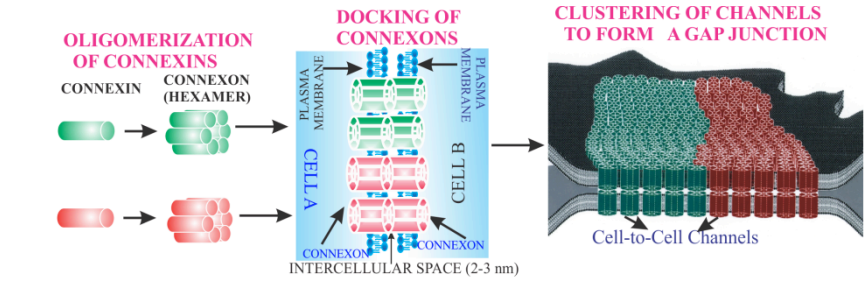

Figure 1. **A cell-cell channel is formed by Cxs, which first oligomerize as hexamers(called connexons or hemichannels), and then are transported to the cell surface and dock with connexons in the adjacent cells to form cell-cell channels. A GJ is formed when several channels cluster at one particular spot. A GJ may be formed of channels formed of more than one Cx. Connexins have 4 transmembrane domains (TM1-TM4 or MI-M4), 2 extracellular loops, and one cytoplasmic domain. The amino and the carboxyl termini face the cytoplasm.**

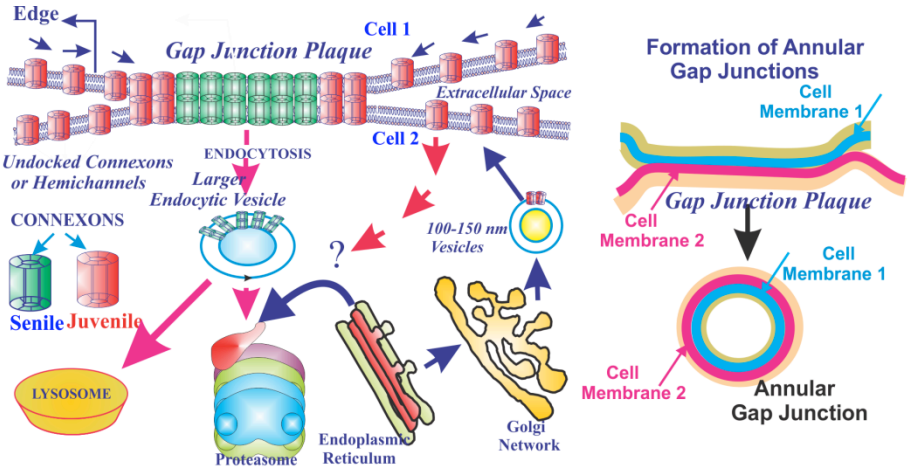

Figure 2. **Assembly and Disassembly of GJs. Cxs are short lived proteins with a half life of 2-5 h. Connexons (see Figure 1) traffic to the plasma membrane (PM) in 100-150 nm particles, diffuse laterally and dock with their counterpart connexons in the PM of apposed cells. Juvenile connexons (red) are recruited to the periphery of the GJ plaque while senile connexons (green) are pinched off from the middle as double membrane vesicles into either one or the other cell. Alternatively, an entire GJ plaque is also endocytosed in its entirety into one or the other cell called annular GJs(left).**

2. Body

Our central hypothesis is that bidirectional signaling between cadherin(Cad)s and Cxs is required to maintain the polarized and differentiated state of epithelial cells and that GJ assembly is the downstream target of the signaling initiated by the classical Cads, with epithelial (E)-Cad facilitating assembly and neuronal (N)-Cad disrupting the assembly. We had proposed 2 specific aims to test this hypothesis:

1. **Determine how E-cad mediated cell-cell adhesion controls the assembly of Cxs into gap junctions in human prostate cancer prostate cancer cell lines.**

2. **Determine the molecular mechanisms by which E-Cad and N-Cad modulate gap junction assembly differentially in human prostate cancer cell lines.**

It is as yet unknown how a bi-cellular structure, such as a GJ, also called a GJ plaque, is endocytosed [1;10-12]. Connexins are short-lived proteins and both the assembly of Cxs into GJs and their disassembly are multi-step processes, which are poorly understood (**Figures 1 & 2).** A GJ can be endocytosed into one or the other cell, either in its entirety, also called an annular GJ, or as fragments pinched off from the center of the plaque as double membrane vesicles, by endocytosis and targeted to the lysosome for degradation. Alternatively, undocked connexons may be endocytosed by clathrin mediated or non-clathrin mediated endocytosis (**Figure 2)** [13-16].

Tasks of Aim 1:

1. Prepare recombinant retroviruses that contain various E-cad constructs that alter its ability to mediate cell-cell adhesion.
    a. Prepare recombinant retrovirus containing E-cad (W156A) (**Johnson**).
    b. Prepare recombinant retrovirus containing E-cad with deleted β-catenin binding site (**Johnson**).
    c. Prepare recombinant retrovirus containing E-cad with mutated p120 catenin binding site (**Johnson**).

2. Generate stable polyclonal cultures of several human PC cell lines (LNCaP - **ATCC**; PC3 - **ATCC**; RWPE1 - **ATCC**; PZ-HPV-7 -**ATCC**) expressing the constructs shown in 1 (**Mehta**).

3. In the cells described in 2, determine if connexins are assembled into gap junctions using Triton X-100 solubility assays (**Mehta and Johnson**).

4. In the cells described in 2, determine if cadherins are assembled into adherens junctions using Triton X-100 solubility assays (**Mehta and Johnson**).

5. In the cells described in 2, observe the trafficking of connexins and their assembly into gap junctions (**Mehta and Johnson**).
    a. Perform cell surface biotinylation to detect connexins at the plasma membrane (**Mehta**).
    b. Determine if connexins co-localize with EEA1, clathrin or caveolin-1 (**Mehta and Johnson**).

6. Knock down endogenous E-cadherin in LNCaP prostate cancer cells (**ATCC**) with or without connexin expression (**Mehta**).

    a. Determine if motility is altered in cells expressing E-cadherin or connexins (**Johnson**).

7. Determine if the trafficking of connexins is altered in knock down cells described in 6 (**Mehta**).

Statement of Work

Aim 1: Determine how E-cadherin mediated cell-cell adhesion controls the assembly of connexins into gap junctions in human prostate cancer cell lines.

Tasks:

1) Prepare recombinant retroviruses that contain various E-cadherin constructs that alter its ability to mediate cell-cell adhesion. (Months 1-9)

- **a) Prepare recombinant retrovirus containing E-cadherinW156A (Johnson).**
- **b) Prepare recombinant retrovirus containing E-cadherinΔβ-cat (Johnson).**
- **c) Prepare recombinant retrovirus containing E-cadherinΔp120 (Johnson).**

Construct E-cadherinW156A in retroviral vector, LZRS, was used to produce recombinant retrovirus. The retroviral construct (20 μg) was transfected in EcoPack cell line and after 48 h the medium containing the virus was collected and filtered (0.45 μM, Millipore, Billerica, MA). To produce recombinant retrovirus for infection of target cells, amphotropic PTi67 cells were infected with the transiently produced recombinant retrovirus from EcoPack and selected in G418 (400 μg/ml). The recombinant retrovirus produced from the pooled polyclonal cultures of PTi67 cells was assayed for the virus titer by colony forming units as described [17-22].

The construct E-cadherinΔβ-cat was prepared as follows: Amino acids 830 through 860 from human E-cadherin, that encompass β-catenin binding site, were deleted (31 amino acid deletion). For engineering the construct E-cadherinΔp120, three amino acid residues (762, 763 and 764) in the cytoplasmic tail of human E-cadherin were mutated to alanines. Thus the sequence was changed from EED to AAA. The construction of these constructs is described [23;24]. We have produced the recombinant retrovirus for E-cadherinΔp120 but not for E-cadherinΔβ-cat.

2) Generate stable polyclonal cultures of several human prostate cancer cell lines (LNCaP - ATCC; PC3 - ATCC; RWPE1 - ATCC; PZ-HPV-7 - ATCC) expressing the constructs shown in 1) (Mehta). (Months 3-12).

We screened several prostate cancer cell lines for the expression E-cadherin and N-cadherin so that we could express the constructs described in task 1. This screen revealed that all the prostate cancer cell lines that we screened expressed E-cadherin or N-cadherin endogenously (**Figure 3**). The characteristics of these cell lines have been described [25-30]. This prohibited us from undertaking the proposed studies in task 2 because the available antibodies for E-cadherin do not distinguish the endogenously expressed E-cadherin from exogenously expressed E-cadherin

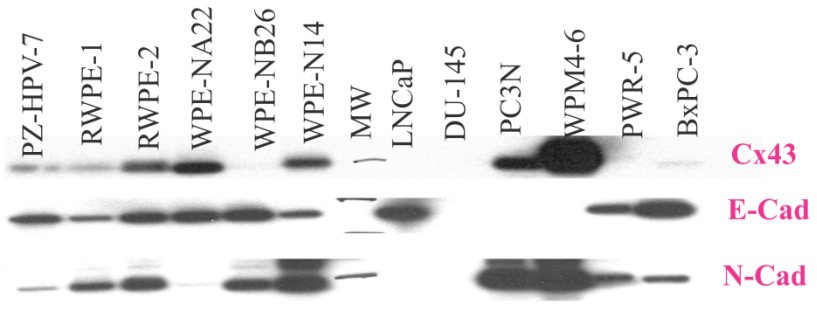

FIGURE 3. We examined the expression of connexins, E-cadherin (E-Cad) and N-cadherin (N-Cad) in all the available, well-characterized prostate cancer cell lines, such as LNCaP, PC3N, and DU-145 as well as in non-transformed cell lines PZ-HPV-7, RWPE-1 and RWPE-2, that have been immortalized by hTERT. All cell lines were from ATCC. WPE-NA22, WPE-NB26, WPE-N14, WPM4-6 and PWR-5 PC cell lines are derived from RPWE-1 after transforming with a chemical carcinogen. BxPC-3 is a pancreatic cancer cell line which expresses E-Cad and N-Cad and was used as a control in these blots. DU-145 cells also express E-Cad, but for reasons not understood, was not detected in this particular blot.

constructs. Introducing these constructs will provide no information with regard to where they are expressed and how they act. These constructs have to be either tagged with Myc or introduced in a prostate cancer cell line that is cadherin null. Alternatively, we will have to design ShRNA for knocking down only the endogenously expressed E-cadherin without knocking down the expression of exogenously introduced engineered E-cadherin constructs. These studies therefore have to be thoughtfully reconsidered and redesigned. One future approach that we would like to undertake is tag both wild type and engineered cadherin constructs with Myc and HA tags, conventionally used by many investigators, to distinguish engineered proteins from endogenously expressed proteins. Highly specific monoclonal and polyclonal antibodies raised against both tags are commercially available and have been used by us in earlier studies [21;23;24;31-34].

The construct E-cadherinW156A, described in task 1a, did not confer cell-cell adhesion when expressed in MiaPaCa cells. These data are significant because they prompt us to undertake similar studies with prostate cancer cell lines that are cadherin null, which we will continue to screen.

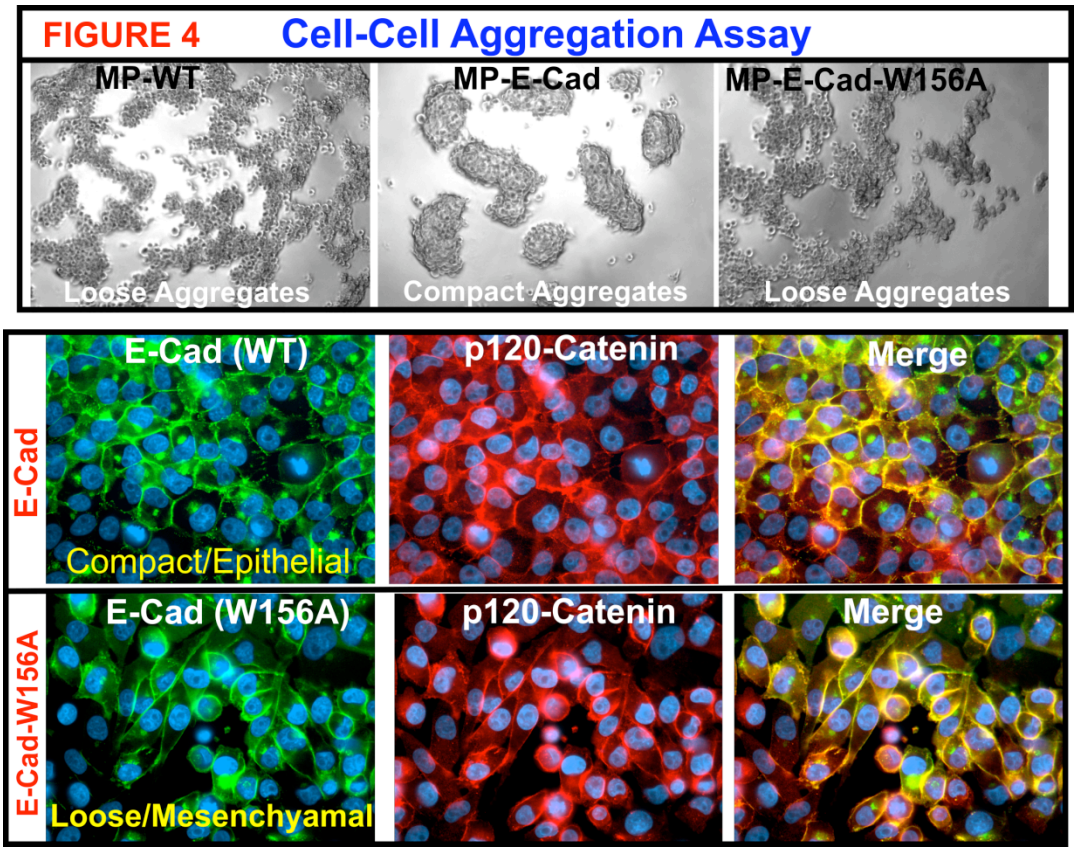

Figure 4. Wild-type (WT) E-cadherin and the mutant E-cadherinW156A (tagged with green fluorescent protein, EGFP) were retrovirally expressed in cadherin-null, human pancreatic cancer cell MiaPaCa. Wild-type and mutant E-cadherin were expressed appropriately at areas of cell-cell contact (**Figure 4, bottom**). As assessed by cell-cell aggregation assays, Cad-null MiaPaCa cells (**MP-WT, Figure 4, top**) and cells expressing mutant Cads (MP-E-Cad-W156A-EGFP) did not adhere and formed loose aggregates, which were dispersed upon trituration (**Figure 4, top**) whereas cells expressing WT E-Cad (**MP-E-Cad, Figure 4, top, middle panels)** formed compact aggregates which could not be dispersed. Both mutant and wild-type Cads were localized at the areas of cell-cell contact **(Figure 4, bottom)**. It is worth noticing that both WT and mutant Cads recruited p120 catenin to cell-cell contact areas (**Figure 4, bottom, middle panel**). In Cad-null MiaPaCa cells, no Cads were seen and p120 catenin was not localized at cell-cell contact areas (data not shown). Cell-cell aggregation assays were performed as described in our earlier studies [18;20].

3) In the cells described in 2), determine if connexins are assembled into gap junctions using Triton X-100 solubility assays (Mehta and Johnson). (Months 4-16)

See comments related to task 2..

4) In the cells described in 2), determine if cadherins are assembled into adherens junctions using Triton X-100 solubility assays (Mehta and Johnson). (Months 4-16)

Please see comments related to task 2.

5) In the cells described in 2), observe the trafficking of connexins and their assembly into gap junctions (Mehta and Johnson). (Months 6-18)
a) Perform cell surface biotinylation to detect connexins at the plasma membrane (Mehta).
b) Determine if connexins co-localize with EEA1, clathrin or caveolin-1 (Mehta and Johnson).

Please see comments related to task 2.

6) Knock down endogenous E-cadherin in LNCaP prostate cancer cells (ATCC) with or without connexin expression (Mehta). (Months 12-20)
a) Determine if motility is altered in cells expressing E-cadherin or connexins (Johnson).

7) Determine if the trafficking of connexins is altered in knock down cells described in 6) (Mehta). (Months 16-24)

We have not initiated these tasks yet.

Aim 2: Determine the molecular mechanisms by which E-cadherin and N-cadherin modulate gap junction assembly differentially in human prostate cancer cell lines.

Tasks:
1) Prepare recombinant retroviruses that contain chimeras of E-cadherin and N-cadherin (Johnson). (Months 6-16)
a) Prepare recombinant retrovirus containing chimeras with the extracellular domains switched (Johnson).
b) Prepare recombinant retrovirus containing chimeras with the cytoplasmic domains switched (Johnson).
c) Prepare recombinant retrovirus containing chimeras with segments of the extracellular domains of E-cadherin and N-cadherin swapped (Johnson).

The preparation of these constructs has been described [31;32]. We have not yet tested these constructs.

2) Infect LNCaP cells (ATCC) and PZ-HPV-7 cells (ATCC) with the retroviruses described in 1) and retroviruses containing wild-type N-cadherin (Mehta). (Months 12-24)

We have not initiated this task. Please see also comments related to task 2 in aim 1.

3) **In the cells described in 2), determine if connexins are assembled into gap junctions using Triton X-100 solubility assays (Mehta and Johnson). (Months 16-28)**

We have not initiated these studies.

4) In the cells described in 2), determine if cadherins are assembled into adherens junctions using Triton X-100 solubility assays (Mehta and Johnson). (Months 16-28)

We have not initiated these studies.

5) In the cells described in 2), observe the trafficking of connexins and their assembly into gap junctions. (Months 24-32)

a) Perform cell surface biotinylation to detect connexins at the plasma membrane (Mehta).

b) Determine if connexins co-localize with EEA1, clathrin or caveolin-1 (Mehta and Johnson).

We have not initiated this task.

6) Determine if N-cadherin alters the motility of connexin-expressing LNCaP (ATCC) and cells PZ-HPV-7 (ATCC) cells (Johnson). (Months 28-36)

This task has not been initiated yet.

7) Determine if N-cadherin induces endocytosis of gap junctions in connexin-expressing LNCaP (ATCC) and PZ-HPV-7 (ATCC) cells (Mehta). (Months 28-36)

We have not yet initiated this study. .

Conclusion:

The available data preclude us to draw any conclusions.

Key Research Accomplishments

The E-cadherinW156A, when expressed in cadherin-null MiaPaCa cell line, failed to induce cell-cell adhesion compared to wild-type E-cadherin.

Reportable Outcomes:

None.

Reference List

1. Laird,D.W. (2006) Life cycle of connexins in health and disease. *Biochem J*, **394**, 527-543.

2. Saez,J.C., Berthoud,V.M., Branes,M.C., artinez,A.D., Bey., and Beyer,E.C. (2003) Plasma membrane channels formed by connexins: their regulation and functions. *Physiol Rev*, **83**, 1359-1400.

3. Crespin,S., Defamie,N., Cronier,L., and Mesnil,M. (2009) Connexins and carcinogenesis. In Harris,A. and Locke,D. (eds.) *Connexinss: A Guide*., pp 529-42.

4. Naus,C.C. and Laird,D.W. (2010) Implications and challenges of connexin connections to cancer. *Nat Rev Cancer*, **10**, 435-441.

5. Plante,I., Stewart,M.K.G., Barr,K., Allan,A.L., and Laird,D.W. (2010) Cx43 suppresses mammary tumor metastasis to the lung in a Cx43 mutant mouse model of human disease. *Oncogene*, **30**, 1681-1692.

6. McLachlan,E., Shao,Q., Wang,H.l., Langlois,S., and Laird,D.W. (2006) Connexisn act as tumor suppressors in three dimensional mammary cell organoids by regulating differentiation and angiogenesis. *Cancer Res*, **66**, 9886-9894.

7. King TJ, Gurley KE, Prunty J, Shin JL, Kemp CJ, and Lampe PD (2005) Deficiency in the gap junction protein connexin32 alters p27Kip1 tumor suppression and MAPK activation in a tissue-specific manner. *Oncogene*, **24**, 1718-1726.

8. King,T.J. and Lampe,P.D. (2004) Mice deficient for the gap junction protein Connexin32 exhibit increased radiation-induced tumorigenesis associated with elevated mitogen-activated protein kinase (p44/Erk1, p42/Erk2) activation. *Carcinogenesis*, **25**, 669-680.

9. King,T.J. and Bertram,J.S. (2005) Connexins as targets for cancer chemoprevention and chemotherapy. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, **1719**, 146-160.

10. Laird,D.W. (2010) The gap junction proteome and its relationship to disease. *Trends Cell Biol*, **20**, 92-101.

11. Berthoud VM, Minogue PJ, Laing JG, and Beyer EC (2004) Pathways for degradation of connexins and gap junctions. *Cardiovasc.Res.*, **62**, 256-267.

12. Musil,L.S. (2009) Biogenesis and degradation of gap junctions. In Harris,A. and Locke,D. (eds.) *Connexins: A Guide*. Springer, pp 225-40.

13. Falk,M.M., Baker,S.M., Gumpert,A., Segretain,D., and Buckheit,R.W. (2009) Gap junction turnover is achieved by the internalization of small endocytic double-membrane vesicles. *Mol Biol Cell*, **20**, 3342-3352.

14. Jordan,K., Chodock,R., Hand,A., and Laird,D.W. (2001) The origin of annular junctions: a mechanism of gap junction internalization. *J Cell Sci*, **114**, 763-773.

15. Piehl,M., Lehmann,C., Gumpert,A., Denizot,J.P., Segretain,D., and Falk,M.M. (2007) Internalization of Large Double-Membrane Intercellular Vesicles by a Clathrin-dependent Endocytic Process. *Mol.Biol.Cell*, **18**, 337-347.

16. Traub,L.M. (2009) Tickets to ride: selecting cargo for clathrin-regulated internalization. *Nat Rev Mol Cell Biol*, **10**, 583-596.

17. Mehta,P., Hotz-Wagenblatt A, Rose B, Shalloway D, and Loewenstein WR (1991) Incorporation of the gene for a cell-to-cell

channel proteins into transformed cells leads to normalization of growth. *J Membr Biol*, **124**, 207-225.

18. Chakraborty,S., Mitra,S., Falk,M.M., Caplan,S., Wheelock,M.J., Johnson,K.R., and Mehta,P.P. (2010) E-cadherin differentially regulates the assembly of connexin43 and connexin32 into gap junctions in human squamous carcinoma cells. *J Biol Chem*, **285**, 10761-10776.

19. Govindarajan,R., Song,X.-H., Guo,R.-J., Wheelock,M.J., Johnson,K.R., and Mehta,P.P. (2002) Impaired traffcking of connexins in androgen-independent human prostate cancer cell lines and its mitigation by α-catenin. *J Biol Chem*, **277**, 50087-50097.

20. Govindarajan,R., Chakraborty,S., Falk,M.M., Johnson KR, Wheelock,M.J., and Mehta,P.P. (2010) Assembly of connexin43 is differentially regulated by E-cadherin and N-cadherin in rat liver epithelial cells. *Mol Biol Cell*, **21**, 4089-4107.

21. Johnson,K.E., Mitra,S., Katoch,P., Kelsey,L.S., Johnson,K.R., and Mehta,P.P. (2013) Phosphorylation on Ser-279 and Ser-282 of connexin43 regulates endocytosis and gap junction assembly in pancreatic cancer cells. *Mol.Biol.Cell*, **24**, 715-733.

22. Mitra,S., Annamalai,L., Chakraborty,S., Johnson,K., Song,X., Batra,S.K., and Mehta,P.P. (2006) Androgen-regulated Formation and Degradation of Gap Junctions in Androgen-responsive Human Prostate Cancer Cells. *Mol Biol Cell*, **17**, 5400-5416.

23. Fukumoto,Y., Shintani,Y., Reynolds,A.B., Johnson,K.R., and Wheelock,M.J. (2008) The regulatory or phosphorylation domain of p120 catenin controls E-cadherin dynamics at the plasma membrane. *Exp Cell Res*, **314**, 52-67.

24. Nieman,M.T., Kim,J.-B., Johnson,K.R., and Wheelock,M.J. (1999) Mechanism of extracellular domain-deleted dominant negative cadherins. *J Cell Sci*, **112 (Pt 10)**, 1621-1632.

25. Miki,J. and Rhim,J. (2010) Prostate cell cultures as in vitro models for the study of normal stem cells and cancer stem cells. *Prostate Cancer and Prostate Diseases*, **11**, 32-39.

26. Miki,J., Furusato,B., Li,H., Gu,Y., Takahashi,H., Egawa,S., Sesterhenn,I.A., McLeod,D.G., Srivastava,S., and Rhim,J.S. (2007) Identification of Putative Stem Cell Markers, CD133 and CXCR4, in hTERT-Immortalized Primary Nonmalignant and Malignant Tumor-Derived Human Prostate Epithelial Cell Lines and in Prostate Cancer Specimens. *Cancer Research*, **67**, 3153-3161.

27. Webber,M., Bello,D., and Quader,S. (1997) Immortalized and tumorigenic adult human prostatic epithelial cell lines: characteristics and application. Part 3. Oncogenes, Suppressor genes, and applications. *Prostate*, **30**, 136-142.

28. Webber,M., Bello D, Kleinman HK, and Hoffman MP (1997) Acinar differentiation by non-malignant immortalized human prostate epithelial cells and its loss by malignant cells. *Carcinogenesis*, **18**, 1225-1231.

29. Webber,M., Bello,D., and Quader,S. (1997) Immortalized and tumorigenic adult human prostatic epithelial cell lines: characteristics and applications Part 2. Tumorigenic cell lines. *The Prostate*, **30**, 58-64.

30. Webber,M., Bello,D., and Quader,S. (1996) Immortalized and tumorigenic adult human prostatic epithelial cell lines :characteristics and applications part 1.cell markers and immortalized nontumorigenic cell lines. *The Prostate*, **29**, 386-394.

31. Kim JB, Islam S, Kim YJ, Prudoff RS, Sass KM, Wheelock MJ, and Johnson KR (2000) N-Cadherin extracellular repeat 4 mediates epithelial to mesenchymal transition and increased motility. *J Cell Biol*, **151**, 1193-1206.

32. Nieman,M., Prudoff,R., Johnson,K., and Wheelock,M. (1999) N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. *J Cell Biol*, **147**, 631-643.

33. Shintani,Y., Wheelock,M.J., and Johnson,K.R. (2006) Phosphoinositide-3 Kinase-Rac1-c-Jun NH2-terminal Kinase Signaling Mediates Collagen I-induced Cell Scattering and Up-Regulation of N-Cadherin Expression in Mouse Mammary Epithelial Cells. *Mol Biol Cell*, **17**, 2963-2975.

34. Shintani,Y., Fukumoto,Y., Chaika,N., Svoboda,R., Wheelock,M.J., and Johnson,K.R. (2008) Collagen I-mediated up-regulation of N-cadherin requires cooperative signals from integrins and discoidin domain receptor 1. *J Cell Biol*, **180**, 1277-1289.

Appendices:

None.

Supporting Data:

None.